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A FIELD-TEST FOR DETECTING ORGANOPHOSPHORUS COMPOUNDS IN WATER

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An enzyme test has been worked out for detecting organophosphorus compounds in water. The test is based on the inhibition of cholinesterase. The detection limits for the »nerve gases« are (μg/L): Soman 0.12, VX 5.9, Sarin 9.9 and Tabun 26. The detection limit for the organophosphorus pesticide dichlorvos is 50 μg/L.

Key terms: chemical warfare agents, cholinesterase inhibition, contamination of drinking water, nerve gases, organophosphorus pesticides

Organophosphorus compounds are used as pesticides, but also as warfare agents (»nerve gases«). Accidental or intentional contamination of the biosphere can therefore easily occur. Potential danger from »nerve gases« in widespread war activities has made it necessary to develop a method to control possible contamination of drinking water supplies under field conditions.

An enzyme test has been chosen to develop a field kit. The test is based upon the kinetics of inhibition of cholinesterases by organophosphorus compounds. The mechanism of cholinesterase inhibition has been known for many years (cf. 1) and many methods for detecting organophosphorus compounds are based on that reaction (e.g. 2).

The rationale of the method is the following: organophosphorus compounds phosphorylate the active site in cholinesterases thereby inactivating the enzyme. The reaction is defined by a rate constant. Knowing the rate constant of inhibition makes it possible to calculate the concentration of a given organophosphate that has caused an observed degree of inhibition under given experimental conditions.

REAGENTS AND PROCEDURE

The following reagents were selected for the field-test: Native human serum was chosen as the enzyme source, because human serum is easily available and serum cholinesterase

(EC 3.1.1.8) is a stable enzyme. Butyrylthiocholine was chosen as the substrate, because it is well hydrolysed by serum cholinesterase. For measuring cholinesterase activity the method of *Ellman and co-workers* (3) was chosen because it is simple and reliable (cf. 4, 5). The method is based upon the reaction of thiocholine with the thiol reagent DTNB (5,5'-dithiobis-(2-nitrobenzoic acid), which gives the yellow anion of 5-thio-2-nitrobenzoate; the appearance of colour can be observed by eye or measured spectrophotometrically. The following reaction times were chosen as suitable for field conditions: incubation of enzyme and organophosphate 15 min, time of assay 5-10 min.

All reagents are supplied in drop-dispensing bottles. Amount per bottle: freeze-dried human serum (20 mg), butyrylthiocholine iodide (48 mg) together with DTNB (6 mg), and Tris/HCl buffer (11 ml, 0.3 M, pH 7.4). Each field kit contains five bottles of each reagent, five graduated tubes, distilled water (100 ml) and a pipette for the distilled water. Before use, serum and substrate/DTNB are each dissolved in 2.0 ml distilled water.

A water sample is tested in the following way: 2.0 ml of a potentially contaminated water sample is placed into a test tube (test sample) and 2.0 ml of distilled water into another test tube (control sample). Twenty drops of buffer and four drops of serum are added into each tube. After 15 min three drops of substrate/DTNB are added and the appearance of yellow colour is observed over 5-10 min. If the test sample contains cholinesterase inhibitors it will be pale yellow or colourless as compared to the control sample which will be intensely yellow.

The final reaction volume during the enzyme assay is 3.0 ml. The final reagents concentrations are: serum 1.6 mg/assay, substrate 2.75 mM, DTNB 0.33 mM and buffer 0.08 M.

DETECTION LIMITS

Using the described procedure, a fifty percent difference in enzyme activity can be well detected by eye. This was concluded by concomitant spectrophotometric measurement of enzyme activity and observation by eye of the colour in the test tube. The detection limit was therefore taken to be the inhibitor concentration that caused 50 per cent inhibition after the chosen time of incubation.

Knowing the rate constants of inhibition of human serum cholinesterase with the »nerve gases« (6) and with dichlorvos (7) the following detection limits were evaluated (μg/L): Soman 0.12, VX 5.9, Sarin 9.9 and Tabun 26. The detection limit for the organophosphorus pesticide dichlorvos was 50 μg/L. These concentrations apply to the reaction at 25 °C in 0.1 M phosphate buffer pH 7.4.

The field kit described in this paper has replaced a field kit which was in use by the army in former Yugoslavia. In the manual of the latter kit the detection limit is quoted as 1-3 μg/L for all »nerve gases« and 500 μg/L for dichlorvos (8). No comparison of detection limits for the two kits is possible, because the manual provides no information concerning the enzyme source and other relevant details.

STABILITY OF THE REAGENTS

The shelf life of the reagents was monitored over a period of one year. The reagents were kept in a laboratory where the ambient temperature reached up to 30 °C during

the summer months. The mean cholinesterase activity in seven different enzyme bottles was 0.199 ΔA/min (range: 0.138-0.248 ΔA/min). The activities varied randomly from bottle to bottle, and the activity range was not related to the time of storage. The same applied to the substrate/DTNB preparations. The mean thiocholine concentration in seven different substrate/DTNB bottles was 3.5 μM (range: 1.3- 7.4 μM) measured in an assay medium of 3.0 ml total volume. It corresponded to only 0.13 per cent of hydrolysed butyrylthiocholine in the substrate/DTNB preparations. Consequently, the shelf life of the enzyme preparation and of the substrate/DTNB preparation is at least one year.

The stability of the enzyme and substrate/DTNB preparation dissolved in water was tested over a period of two weeks, by keeping the solutions at ambient temperature. The enzyme activity remained unchanged. The thiocholine concentration increased from 3.5 μM to 5.1 μM (range: 2.3-11 μM) in solutions from seven different substrate/DTNB bottles. Consequently, both reagents could be used for at least two weeks after being dissolved in distilled water.

COMMENTS

This test has been checked in the field by the Croatian health service laboratories responsible for the control of drinking water supplies. The field-test has been in use since late 1991. The test is applicable not only to warfare agents, but to all direct cholinesterase inhibitors. Many pesticides, such as the organophosphate dichlorvos and the carbamate carbaryl, can be detected by this test. It is therefore expected that the test will also find application in agriculture and health services.

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Sažetak

TERENSKA METODA ZA DOKAZIVANJE ORGANOFOSFORNIH SPOJEVA U VODAMA

Razradena je terenska metoda za dokazivanje organofosfornih spojeva u vodama, koja se osniva na kinetici inhibicije kolinesteraza. U metodi se rabe sljedeće reagencije: lifolizirani serum čovjeka kao izvor enzima, butirilтiokolin kao supstrat i DTNB kao tiolni reagents. Garnitura test-reagencija sadržava i pufer i destiliranu vodu. Enzim i potencijalno kontaminirana voda inkubiraju se 15 min prije dodatka supstrata, a zatim se tijekom 5-10 min prati nastajanje žute boje. Ako je voda kontaminirana organofosfornim spojevima, onda će otopina biti bezbojna ili svijetložuta u odnosu na kontrolni uzorak, koji će imati intenzivno žutu boju. U kontrolnom uzorku enzim se inkubira s destiliranom vodom. Granice detekcije za nervne bojne otrove su (μg/L): Soman 0,12, VX 5,9, Sarin 9,9 i Tabun 26. Granica detekcije za organofosforni pesticid diklorvos: 50 μg/L.

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Ključne riječi: inhibicija kolinesteraze, kontaminacija vode za piće, nervni bojni otrovi, organofosforni pesticidi